AN EVALUATION OF THE POSSIBLE DETRIMENTAL EFFECTS BY THE INTRODUCTION OF ORGANIC AND SECOND-ORDER ORGANICS ON COMMERCIAL AND SPORT FISHING IN LAKE SUPERIOR

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ABSTRACT

The 11,500 square acre St. Louis Estuary of Lake Superior is an interesting aquatic ecosystem. The harbor and estuary are vitally important to at least thirty-six estuary or lake-dwelling fish species as well as a variety of birds, plants, and other animals. The St. Louis River and Estuary is the largest river system flowing into Lake Superior and was recognized by the Indians as the prime spawning grounds for the Walleye Pike (Stizostedion vitreum vitreum), Northern Pike (Esox lucius), and Yellow Perch (Perca flavescens) supplying Western Lake Superior. The Walleye Pike especially is sought after by both commercial and sport fishermen, since it is known for its tender and tasty fillets.

Fishing started to wane in the early 1900s, not owing to a cessation of the spring migratory run of walleyes from Lake Superior, but owing to "pollution." The harmful pollution in this instance was, to a significant extent, the effluent from the new paper mill operations about 33 Km upstream from Lake Superior on the St. Louis River in the village of Cloquet, Minnesota. The mid-summer dissolved oxygen levels (caused by the nutrient and biological oxygen demand loadings from organic material) rapidly dropped to levels too low for resident fish. The result was that fish either left the estuary to return to Lake Superior or, in certain instances, died of suffocation. The river furthermore acquired a chemical odor associated with the chemical nature of the pulping process of the paper mill operation, and fish fillets rapidly acquired taste and odor qualities associated with the variety of chlorophenolic products produced in the chlorination bleaching process used for the whitening of paper. Fishermen turned their attention to the bountiful inland lakes of northern Wisconsin and Minnesota, and the fishing potential of the St. Louis Estuary was largely neglected for seventy years.

The question of pollution in this estuary is, of course, certainly more complex than a problem associated with just chloroorganics and taste impairment in the flavor of fish. It reflects the complex nature of the municipal, industrial, agricultural, airborne, and natural causes of degradation of water quality. Nevertheless, it was chosen as the focus point of this investigation because the recent dramatic change in the St. Louis Estuary, brought about by a regional concept for advanced waste water treatment, has had its greatest visual impact in the remarkable recovery of sport fishing in this area. This return is in a large part attributable to the improved taste of walleye fillets, associated with lower levels of dissolved chloroorganics and the higher dissolved oxygen levels found in the river, resulting in an improved fish habitat.

The rapidity and extent of the river cleanup was, and continues to be, difficult to evaluate in an exact and unambiguous fashion. Certainly, quantitative physical and hygienic water quality standards can be measured routinely, and aesthetic qualities, such as odor and appearance, can be readily observed. In the present investigation, both a survey

of fishermen and a controlled fish taste panel agreed that the palatability of walleye pike caught in the St. Louis Estuary has improved.

The critical determination of trace levels of chloroorganics, however, is much more difficult and yet is one of the most important factors relating to the recovery of the St. Louis Estuary as a viable fish habitat. Furthermore, the diversion of the paper mill effluents to the downstream WLSSD facilities does not necessarily imply the levels of chloroorganics will go to zero as this must be related to the "flushing rate," i.e., the time required to wash out the contaminants. Even more critical to the present investigation is the evaluation of the efficiency of the downstream WLSSD treatment plant in reducing the actual chloroorganic level previously released directly into the river by the paper mill operation. The results of the present study, in fact, show that certain chlorophenols survive the biological treatment process and continue to be released into the headwaters of the St. Louis Estuary.

This is consistent with chloroform monitoring of the St. Louis Estuary and the paper mill and WLSSD effluents as an "indicator" of the chloroorganic problems. The chloroform monitoring concept offers several advantages. First, chloroform is known to form in the chlorination of wood products and changes in chloroform levels can be expected to correlate in a qualitative fashion with the total chloroorganic production. Second, we can routinely analyze for chloroform in a sensitive and reliable fashion and thus avoid the complicated and expensive analysis of a complex matrix of chloroorganics.

The chloroform monitoring in the waters of the St. Louis Estuary correlates very well with the fish palatability studies before and after the start-up of the WLSSD operation. Furthermore, the dramatic increase in chloroform concentrations in the WLSSD effluent, as compared to the old municipal treatment plant, suggests that large amounts of chloroorganics are still being released in the estuary, albeit at the headwaters rather than upstream in the prime fishing areas.

The need to analyze for phenolic components in a sensitive fashion has led to the development in this study of a new technique for the analysis of phenols in natural waters. The analytical procedure utilizes 2-fluorenyl sulfonyl chloride and the labeled compounds are subsequently analyzed by high-performance liquid chromatography (HPLC) employing fluorescence detection. Use of the fluorenyl derivatives and fluoresence detection has lowered the detection limits about fifty times over conventional UV detection systems. A typical phenol determination requires only about two hours.

CONCLUSIONS/APPLICATIONS

The present study shows that the improvement of sports fishing in the St. Louis River of Lake Superior, because of the improved taste and odor properties of fish caught in this river system, is consistent with a decreased level of solubilized chloroorganics in the spawning regions of the river owing to the diversion of the Potlatch Paper Mill effluent to the new Western Lake Superior Sanitary District treatment plant located at the mouth of the estuary.

The present study has developed two novel approaches to the analysis of a complex matrix of chloroorganics such as that associated with the St. Louis River's regional sewage treatment plant handling industrial paper mill wastes. One approach is to use an "indicator analysis" as a monitor of the total problem. In the present investigation it was found that chloroform appears to be a good indicator compound of chloroorganic problems arising from a paper mill operation using chlorine in the bleaching process. The second approach was the development of a fast and yet sensitive method to analyze for various phenols, the major odor/taste problem-causing compounds. In the present procedure the use of a "fluorescent label" was developed and coupled with High-Performance Liquid Chromatography (HPLC). The method offers a sensitive and selective way to analyze complex environmental samples.

It is hoped the results obtained here can be applied to related ecosystems, especially to the value and limitations of using an advanced secondary biological treatment process to handle paper mill effluents.

MATERIALS/METHODS

Materials

Chloroform, bromodichloromethane, carbon tetrachloride, bromoform, phenol, 2,4- dichlorophenol, 1,2,4,-trichlorophenol, 2,3,4,5-tetrachlorophenol, and pentachlorophenol, were purchased from Aldrich Chemical Company. Hexane, isooctane, methylene chloride, cyclohexane, methanol, and acetone were obtained from Burdick and Jackson Laboratories (pesticide grade redistilled). Sodium sulfate, sulfuric acid and fuming sulfuric acid were obtained from Mallinckrodt Chemical Company. Celite 545 was obtained from Baker Chemical Company.

Procedure for Chloroform Analysis

Sampling. All samples taken were surface samples collected in 150 ml glass bottles and sealed with teflon-silicone septa and aluminum seals so as not to leave any headspace. (Pierce Co., Rockford, Ill.) Samples were stored at 4° C until analysis.

Glassware. All glassware and sample bottles were cleaned with chromic acid cleaning solution, rinsed with tap water, distilled water, acetone, isooctane, dried in oven, and covered with aluminum foil until used.

Standards. Stock standards containing chloroform, bromodichloromethane, and bromoform were prepared gravimetrically by micropipeting the THMs into isooctane.* Mixed standards containing two, three, and all four of the THMs were also made. Working standards containing the THMs were prepared by diluting the stock solution with isooctane. Standards of THMs were also made in methanol for determination of the solvent extraction efficiency.

Extraction Procedure. The extraction solvent (isooctane) was added directly to the sampling bottle using two 10 ml syringes; with 22-gauge needle. One syringe contains the solvent; the other is empty. The needles of both syringes were pierced through the septum, and 10 ml of solvent was injected into the bottles. The solvent displaced the water, which was collected in the second syringe and discarded. The partioning was then brought to equilibrium by shaking for 15 minutes. Then, an aliquot (2-4 µl) of the organic layer was removed with a microliter syringe for chromatographic analysis.

Preparation of Standard Curve. Duplicate 2 µl injections of each working standard (5 to 200 ppb) were made. Peak heights were measured in mm and plotted against concentrations in ppb. The range of standards was chosen to include the normal range of concentrations found in the water samples. The response as a function of concentration was linear throughout the range of the working standards (Figure 1).

Extraction Efficiency. The extraction efficiency of isooctane was determined by spiking lake water with chloroform standards in methanol.

Apparatus. A Hewlett-Packard 5733 A gas chromatograph (GC) equipped with a ^{63}Ni electron capture detector was used for analysis. The column was 6 ft x 2 mm ID glass, packed with 10% squalene on 80-100 mesh Chromasorb W/AW. The analysis was run isothermally at 70°C with 95% Argon-5% methane as the carrier gas at a flow rate of 25 ml/min. The attenuation is normally set at X32.

*Note: The method described was shown to work for the analysis of all THMs. However, this paper will deal only with chloroform levels. In previous work, $CHBr_2Cl$ and $CHBr_3$ were not detected and $CHCl_2Br$ was found only in WLSSD plant samples.

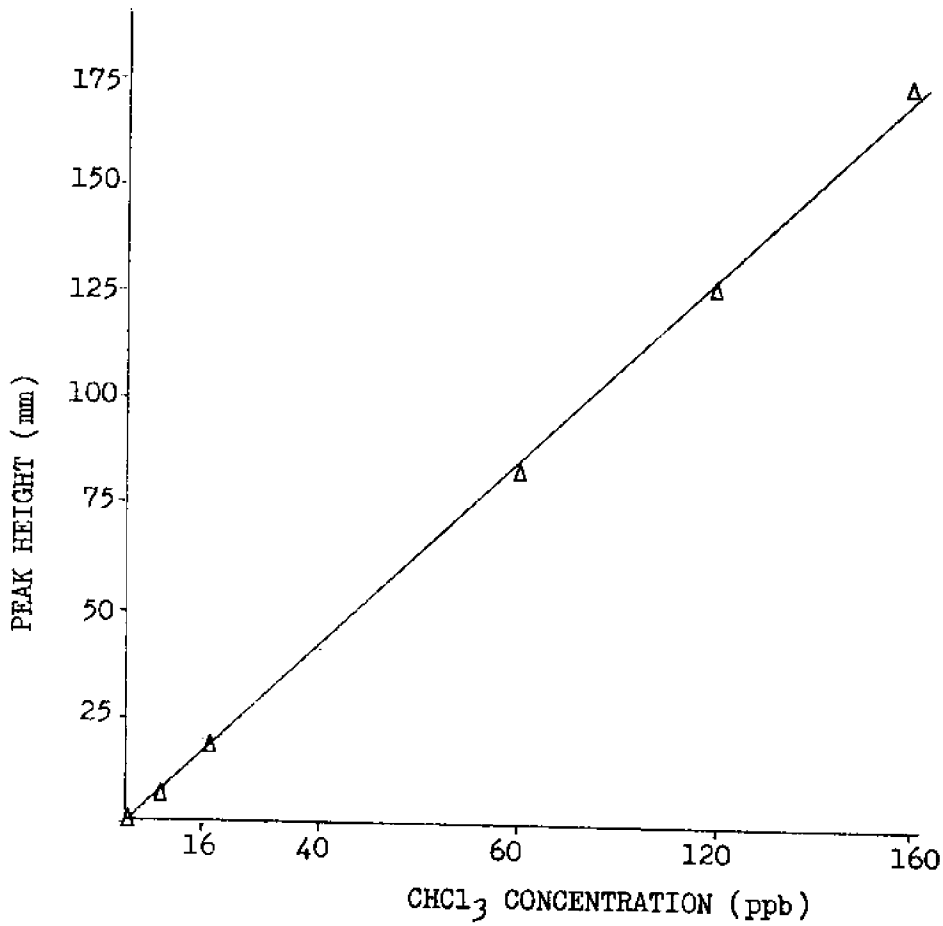


Figure 1 Plot of peak heights versus concentration for chloroform standards.

Procedure for the Analysis of Fish for Chloroorganics

Soxhlet Extraction

The fish were homogenized in a blender and 100g aliquots were dried by mixing with sodium sulfate (3g $NaSO_4$/1g fish). The mixture was extracted overnight with hexane-methylene chloride (1+1) in a large Soxhlet extractor.

Gel Permeation Chromatography (GPC)

The tissue extract was concentrated to less than 50 ml in methylene chloride and centrifuged to remove particulates. The lipid residue solution was placed on a 2.5 cm by 50 cm SX-2 (Bio-Rad Laboratory) gel permeation column and eluted with methylene chloride at 3.5 ml/min. An automated GPC system developed by Kuehl and Leonard (in press), was used. After the first injection, the waste timer was adjusted so that the

sample collection coincided with the end of elution of lipids as shown in the chromatograph in Figure 2. A Varian UV detector was used to monitor the column effluent.

The collected fraction in methylene chloride was concentrated to 1 ml in a Kudnerna-Danish apparatus with a 3-ball Synder column. The sample was further cleaned up by chromatographing on a column containing 15g Celite 545 impregnated with 9 ml of a 1:1 mixture of H_2SO_4 and fuming H_2SO_4 (30% SO_3) and eluting with 150 ml hexane. The extract was concentrated to 1 ml.

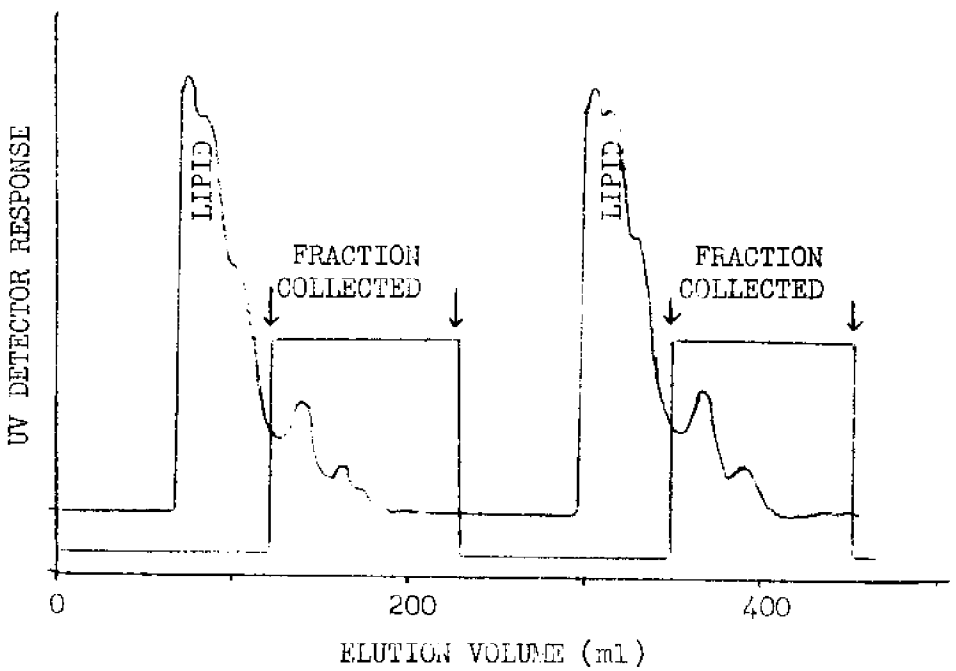


Figure 2 UV chromatogram of GPC effluent (Fond du Lac fish fillet spiked with chlorophenols).

Derivatization

The sample was derivatized with diazoethane, prepared by using a Diazoethane generator (minimole size, Pierce No. 28131) and the method reported by Fales, Judini, and Babashak (1973).

0.14 g of N-Ethyl-N'-nitro-N-nitrosoguanidine was placed in the inside tube through its screw cap opening along with about 1 ml of distilled water to dissipate any heat generated. 3 to 4 ml Hexane was placed in the outside tube and the two parts were clamped together. The lower part was immersed in an ice bath and about 0.6 ml of 20% KOH was

injected through the rubber septum. About 45 minutes were allowed for the gas to collect in the cold hexane. The hexane, saturated with diazoethane, was pipeted into the sample at approximately 0.1 ml per 1 ml of sample (Rivers, 1972).

After derivatization the sample volume was adjusted to 5 ml with hexane. The sample, 0.5 ml of the 5 ml, was diluted to 25 ml with hexane for analysis. (See Figure 3)

Electron-Capture Detector Analysis

Analysis of the 1-50 dilutions of the 5 ml fish extract were performed on a Hewlett-Packard Model 5700A gas chromatograph equipped with a Model 3352B Laboratory Data System, an automatic sampler, and a linearized argon-methane detector. Instrument parameters and operating conditions follow:

Column: glass 6 ft x 1/8 inch ID, packed with 80-100 mesh Gas Chrom Q coated with a mixture of 4% SE-30 and 6% OV-210

Column Temp: programmed from 180 to 220° at 4° C/minute followed by an 8-minute hold at 220°C for PCBs. programmed from 80-220 at 4° C/minute followed by an 8 minute hold at 230°C for chlorophenols

Carrier Gas: A mixture of 90% argon and 10% methane flowing at 30 ml/minute.

The chromatograms were interpreted by a computer program for PCBs (Veith, et al., 1976). Chlorophenols were determined by retention times compared to standards.

GC/MS Analysis

The analyses were performed on a GC/MS system consisting of a Varian Aerograph Model 1700 gas Chromatograph and a Varian MAT CH-5 single focusing mass spectrometer.

The Varian MAT CH-5 mass spectrometer is interfaced to a Finnigan Incos 2300A data system equipped with a Tetronix 2040 terminal and a Versatec Model 80 electrostatic printer/plotter.

Instrument parameters and operating conditions were as follows:

Column: glass, 6 ft x 1/8 inch ID, packed with 80-100 mesh Gas Chrom Q coated with 3% OV-101

Column Temp: 25-225 C at 4° C/minute

Column Flow:	30 ml/min of He
Source Temp:	375° C
Mass Scan:	50-550
Electron Energy:	70 ev
Resolution:	1000

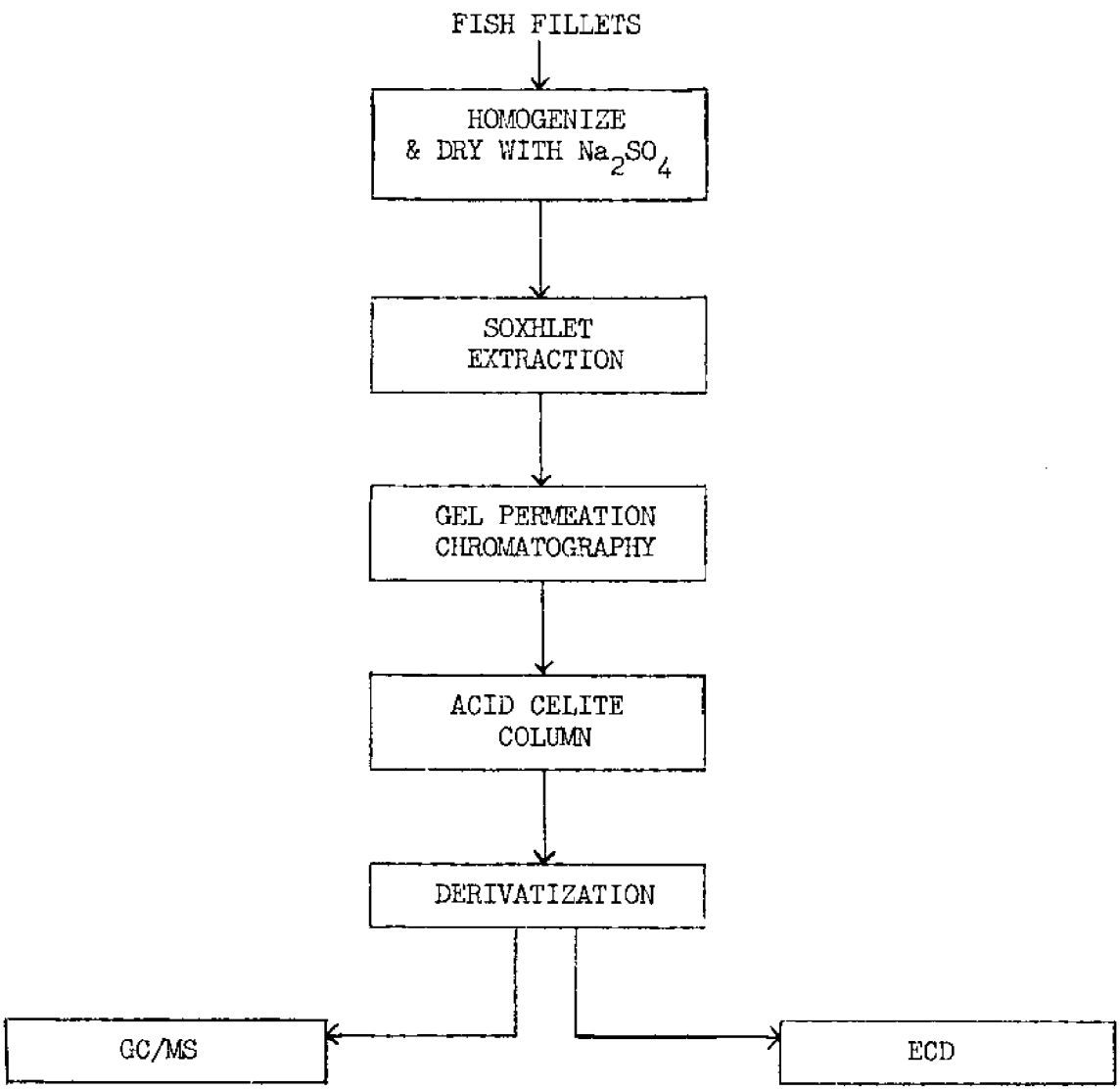


Figure 3 Schematic of the analytical procedure used for the identification of chlorinated organics in fish.

Analysis of Water Samples

Procedure. Water samples were collected in glass bottles and refrigerated at 4° C until analyzed. For a description of sampling sites see map (Appendix A). A schematic of the procedure used for analyses is shown in Figure 4.

Steam Distillation. By means of the steam distillation method outlined by Veith and Kiwus (1977), the water samples were acidified to pH 2 with concentrated H_2SO_4. The chloroorganics were steam distilled into isooctane for 2 hours. All glassware was solvent washed before use and blanks determined before each sample run. The isooctane extracts were dried over anhydrous Na_2SO_4 and concentrated to 1 ml.

CsOH/Silica Gel Chromatography. Using a method published by Ramljak (1977) and refined by Stalling et al. (1978), the isooctane extract from steam distillation was placed on a 5 mm x 25 mm column of silica gel treated with cesium hydroxide prewashed with methylene chloride/cyclohexane (1:1 v/v).

The non-polar chloroorganics were eluted with 10 ml hexane concentrating to 1 ml. The acidic fraction was eluted with 10 ml of methanol, concentrated to 1 ml and diluted with 5 ml of distilled water acidified to pH 2. The acidified mixture was then extracted 4 times with 4 ml portions of hexane. The hexane extract was dried over anhydrous Na_2SO_4 and concentrated to 1 ml. The acidic fraction was then derivatized with diazoethane as previously described.

GC/MS and EC/GC analyses were performed as previously described. For GC/MS samples with low concentrations it was necessary to concentrate the acidic fraction to 0.1 ml and make a 25 ul injection.

Sediment Analysis

Procedure. The possibility of chloroorganics being absorbed in bottom sediments in the estuary was investigated at the WLSSD site. A schematic of the procedure is shown in Figure 5.

Sampling. Sediment was collected using an Echmann dredge. The sediment samples were stored in solvent washed glass jars and frozen until analysis.

Analysis. 50 g of the air-dried sediment were blended with distilled water (acidified to pH 2 with H_2SO_4) and added to a 1000 ml distilling flask. A teflon coated stirring bar was added and the sample mixture was boiled over a hot plate stirrer for 2 hrs using the steam-distillation unit developed by Veith and Kiwus (1977). The isooctane extract was collected and analyzed following the procedure outlined for water analysis.

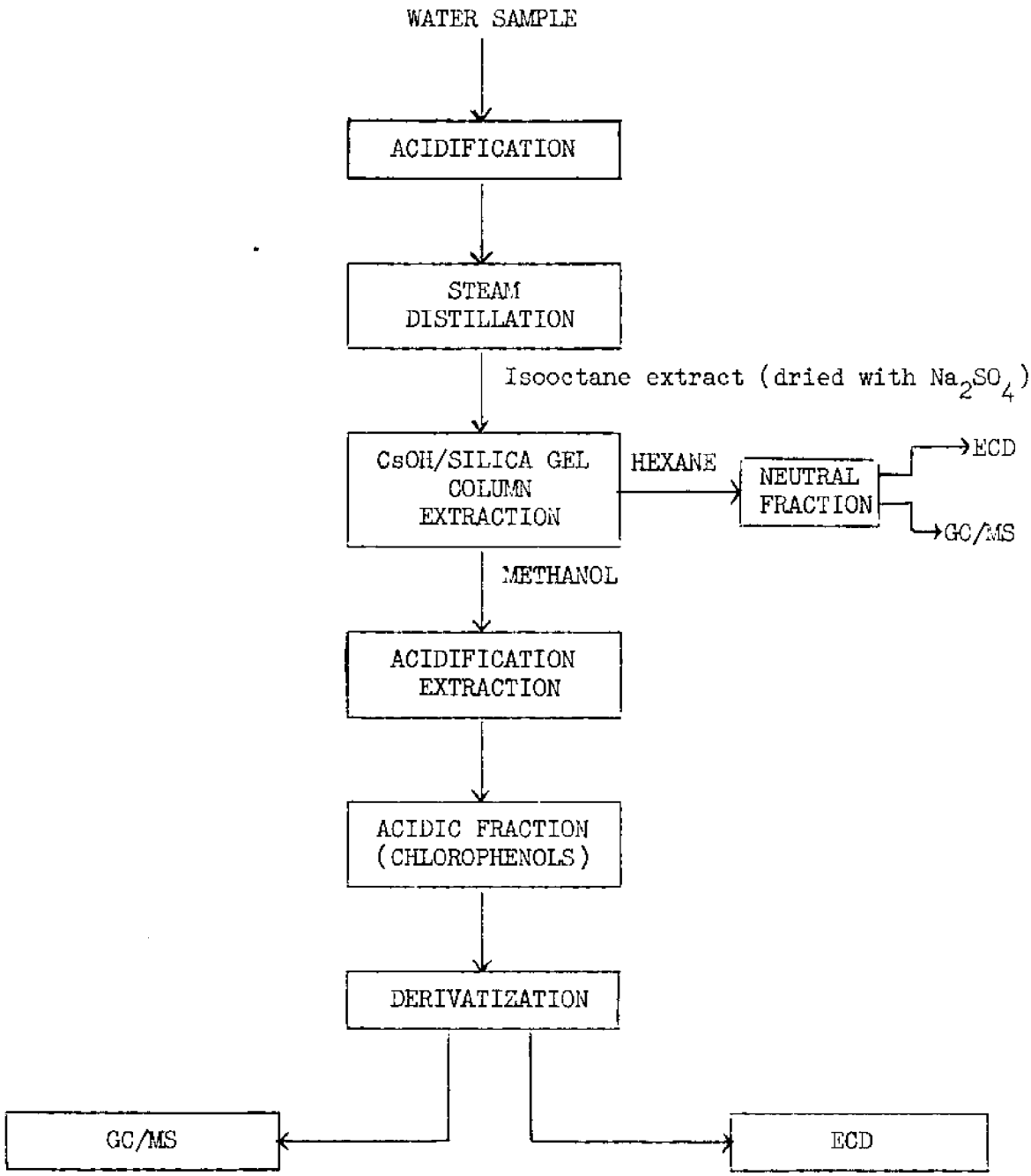


Figure 4 Schematic of the analytical procedure used for the identification of chlorinated organics in water.

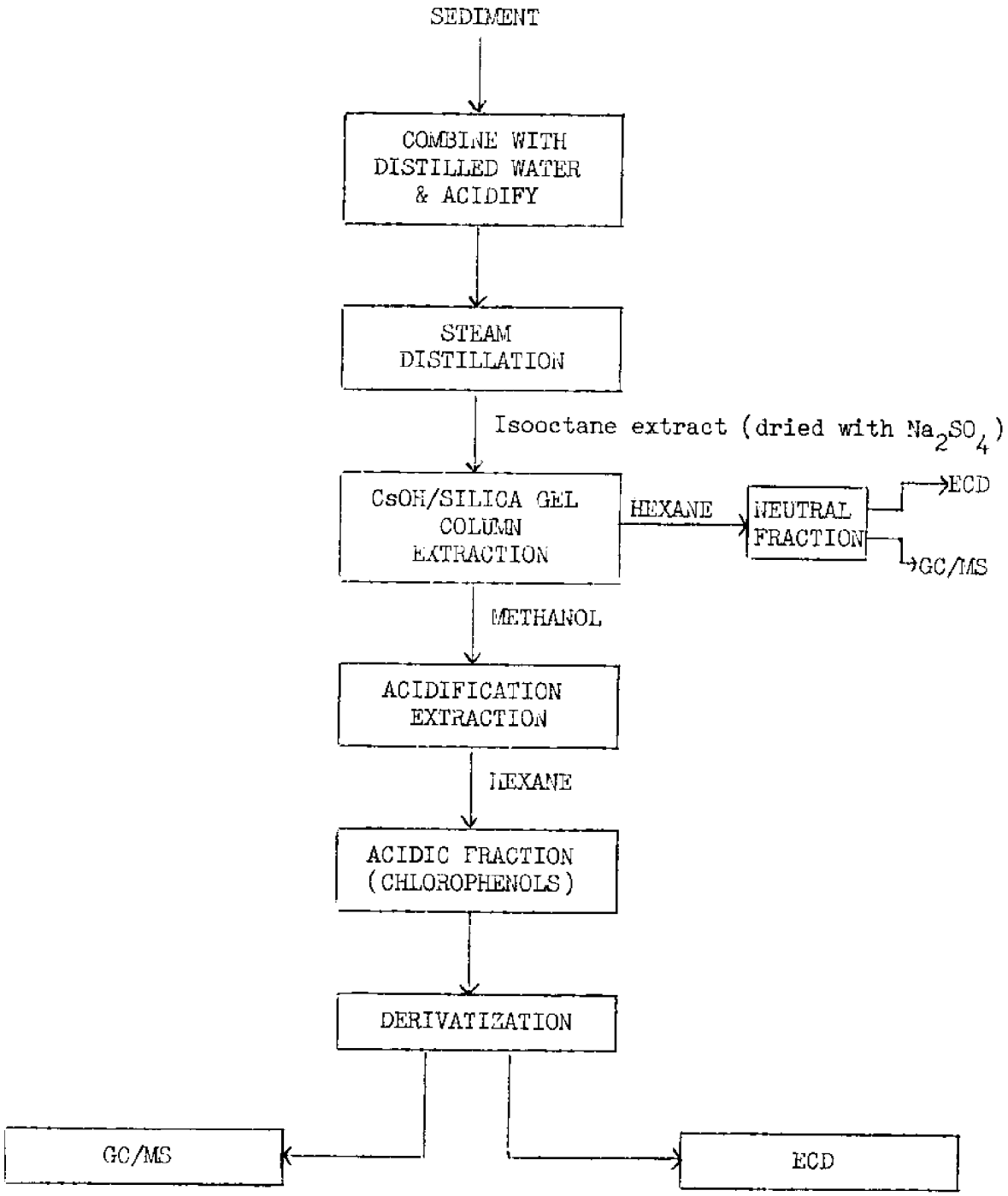


Figure 5 Schematic of the analytical procedure used for the identification of chlorinated organics in sediment.

RESULTS AND DISCUSSION

INTRODUCTION

No doubt the most dramatic change in water quality in the Lake Superior watershed has been the clean-up of the St. Louis Estuary with the start-up of the Western Lake Superior Sanitary District (WLSSD) sewage treatment plant. The total concept approach for this plant combines advanced waste water and solid waste disposal in a single sophisticated system for an entire region. This advanced regional treatment project, which serves a 1,300 square kilometer area in northeastern Minnesota, was built at a cost of $108 million and started operation in the winter of 1978-'79.

Wastewater entering the plant is treated biologically by an activated sludge system where bacteria in the wastewater are put to work consuming organic material and nutrients. The wastewater is further treated by a chemical flocculation process to remove phosphorous and then filtered through mixed media filters to remove fine particulates. The effluent is chlorinated to kill bacteria before being released to the headwaters of the St. Louis Estuary.

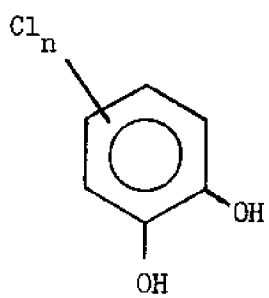
The treatment facility to date has met or exceeded many expectations. It treats an average of about 160 million liters per day and removes more than 300 metric tons of pollutants per day, pollutants that earlier were dumped into the river. The plant effluent consistently exceeds federal requirements for biochemical oxygen demand (BOD), suspended solids, and phosphorous. The activated sludge system has proven to be an amazingly resilient system and has been able to withstand shock loads from industrial sources such as the paper mill operation in Cloquet.

It is especially significant to the present report that the Potlatch paper mill effluent is now treated at the new WLSSD facility rather than being dumped directly into the river. The effluent from the paper mill receives a primary treatment by clarifiers at the plant to reduce the loading received by the WLSSD plant with its more advanced and expensive secondary treatment process. The sludge from this primary treatment is disposed of in a landfill near Cloquet.

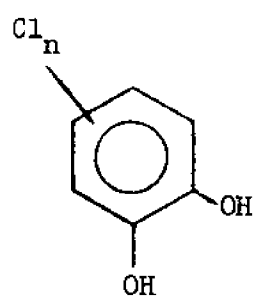
It was thus anticipated that the regional WLSSD advanced treatment plant, coupled with the pre-treatment clarifiers at the Potlatch paper mill operation in Cloquet, would greatly enhance the water quality in the St. Louis Estuary of Lake Superior. However, the rapidity and extent of the river cleanup was, and continues to be, difficult to evaluate in an exact and unambiguous fashion. Certainly, quantitative physical and hygienic water quality standards can be measured routinely; and aesthetic qualities, such as odor and appearance, can be readily observed. The critical determination of trace levels of chloroorganics,

however, is much more difficult and yet is one of the most important factors relating to the recovery of the St. Louis Estuary as a viable fish habitat. Furthermore, the diversion of the paper mill effluents to the downstream WLSSD facilities does not necessarily imply the levels of chloroorganics will go to zero as this must be related to the "flushing rate", i.e., the time required to wash out and/or degrade the contaminants. The flushing rate will depend to a large extent upon the rate at which the bottom sediments lose their trapped and absorbed pollutants to the aqueous environment. It is not known whether this process will take several years, or even decades, but it certainly is not instantaneous. But can one measure the change in trace quantities of chloroorganics expediently? In fact the routine analysis of a complex matrix of chloroorganics is prohibitively complicated and expensive, especially for a long term study, reflecting the analytical methodology required for an exhaustive analysis.

This is further complicated by the need to evaluate the efficiency of the WLSSD treatment plant in reducing the chloroorganic load from the Potlatch Paper Mill operations. It has been well established that biological treatment of papermill effluent is effective in reducing only certain types of the chlorinated phenols produced in the bleaching process. Chlorocatechols, for example, appear to be consumed more readily than chloroguaiacols. The effectiveness of the present WLSSD plant in reducing paper mill chloroorganics has never been established



Chloroguaiacols
n=1-4



Chlorocatechols
n=1-4

and the clean-up of the St. Louis River may simply reflect, in part, the diversion of the point source of chloroorganics downstream to the location of the WLSSD plant (see map in Appendix).

Any attempt to examine the chloroorganic load of the WLSSD effluent must also consider that the treatment plant itself uses large quantities of chlorine year-around to ensure the destruction of pathogenic organisms associated with domestic sewage. This rather dubious disinfection requirement ensures the production of additional chloroorganics.

This report discusses our approaches to evaluating the rehabilitation of the St. Louis Estuary as well as long-range ramifications of the WLSSD operation. The procedures involve a combination of field studies to evaluate "fish quality" in the estuary as well as select chloro-organic chemical analyses that should relate to the taste and odor problems observed in the estuary prior to the start-up of the WLSSD plant.

Source of the Chloroorganic Problem

Of the chlorine produced in the U.S., 15% is used by the pulp and paper industry for bleaching (Chem. Eng. News, 1979). Experimental results (Hardell, de Soura, 1977),(Lund et al., 1979) indicate that about 10% of the applied chlorine is incorporated in non-volatile organic compounds dissolved from the pulp, with considerably more occurring in the volatile organics such as chloroform.

Pulp bleaching is conventionally carried out in five or six sequential stages in which lignin is oxidized and depolymerized using chlorine, chlorine dioxide, and sometimes hypochlorite. Lignin fragments are extracted with sodium hydroxide. The pulp is washed after each stage to remove soluble material and residual bleaching chemicals with effluents from the first two stages containing most of the lignin and chlorinated organics extracted during bleaching. The pulping, bleaching and chemical processes used by the Potlatch Corporation of Cloquet, Minnesota use about 55,550 lbs/day of chlorine (in the form of Cl_2 gas, sodium hypochlorite, and chlorine dioxide, and its total effluent accounts for approximately 40% of WLSSD's daily load.

A literature search shows that extensive work has been carried out to identify the chloroorganic constituents of bleach plant effluents. For example, (Ball et al., 1978) investigated spent bleach liquors from the bleach plant of some Kraft pulp mills for their content of chlorophenols. They found that three types of chlorinated phenols predominate: chloroguaiacols, chlorocatechols, and chlorophenols. A list of the acidic chloroorganics identified in this study is given in Table 1.

TABLE 1

SUMMARY OF ACIDIC CHLORINATED ORGANIC COMPOUNDS THAT HAVE BEEN IDENTIFIED IN PULP AND PAPER MILL EFFLUENTS

Compound
Dichloroguaiacol
Trichloroguaiacol
Tetrachloroguaiacol
Chlorodehydroabietic Acid
Dichlorodehydroabietic Acid
9,10-Dichlorostearic Acid
9,10-Epoxystearic Acid
Chloromalealic Acid
Chlorofumaric Acid
Chlorophenol
Dichlorophenol
Trichlorophenol
Tetrachlorophenol
Pentachlorophenol
Trichlorodimethoxyphenol
Chlorotrihydroxybenzene
Chlorosyringaldehyde
Chloropropiovanillone
Dichlorocatechol
Trichlorocatechol
Tetrachlorocatechol
Chloroethylcatechol
Dichloroethylcatechol
Cichloropropylcatechol
Dichlorobutylcatechol
Tetracholro-o-benzoquinone

TABLE 2 (Clays, 1980)

TOXIC CHLORINATED CONSTITUENTS IN BLEACHED KRAFT MILL EFFLUENT

Compound	Effluent[a] Stream	Effluent Loading[b] (g/metric ton pulp) Untreated	Biotreated	Range of 96-hr[c] LC50 (mg/l)
Trichloroguaiacol	KE	6.0	5.4	0.7-1.0
Tetrachloroguaiacol	KE	4.3	4.7	0.2-0.4
Trichlorocatechol	KC	3.6[d]	ND[e]	1.0-1.5[f]
Tetrachlorocatechol	KC	2.5[d]	ND	1.5[f]
Monochlorodehydroabietic Acid	SC,KE	12.5	0.7	0.6-0.9
Dichlorodehydroabietic Acid	KE	4.7	0.4	0.6-1.2
Dichlorostearic Acid[h]	KE	30	0.1	2.5

[a]KE, kraft mill caustic extraction effluent; KC, kraft mill chlorination-stage effluent; SC, sulfite mill chlorination-stage effluent.

[b]See (Lindstrom, 1978)

[c]See (Dryssen, 1978)

[d]See (Eklund, 1978)

[e]Not detected

[f]See (Keith, 1976)

[g]Principle chlorinated toxicant at sulfite mills

[h]See (Leach, 1975)

Of all organic pollutants affecting the taste and odor of drinking water, phenols have probably been the most widely studied. Maximum taste-producing potential develops after partial chlorination of these compounds. Undesirable flavor or odors often result following the conventional chlorination of waters containing extremely low concentrations of phenolic compounds. The U.S. EPA limit of 0.001 mg/liter phenol in drinking water is an aesthetic standard based on the fact that formation of chlorophenols at concentrations above approximately 0.005 mg/liter results in a characteristic medicinal taste. Toxicities of some of the various chlorinated compounds identified in bleached kraft mill effluents are shown in Table 2 (Peterman et al.). Two of these compounds, trichloro- and tetrachloroguaiacol were taken up by perch and pike caught in the receiving water of a Swedish bleached Kraft mill and by rainbow trout exposed to effluent under laboratory-controlled conditions. (Landner, 1973).

The presence of chloroform in bleach pulp effluents was first mentioned by Harris, et al., in 1934. The National Council of the Paper Industry for Air and Stream Improvement (NCASI) analyzed effluent samples, before and after biological treatment, from nine pulp mills practicing bleaching (NCASI, 1977). Chloroform, produced at an average of 0.7 lb/ton of dried pulp production, was detected and found to be formed mainly during the hypochlorite stage of bleaching.

CHLOROFORM MONITORING

As indicated above, the primary point source of chloroorganics in the St. Louis River is known to be the bleaching process of the Potlatch paper mill operation. The other major contributor to chloroorganic levels is the chlorination process of the WLSSD treatment plant itself. Long-term monitoring of chloroorganic levels in the river, therefore, is highly complex and expensive owing to the vast variety of compounds formed. Chloroform is known to be formed in both the chlorination of waste water and during the pulp bleaching process. Thus, the use of chloroform as a possible indicator species for the monitoring of the total chloroorganic problem was examined, (i.e., higher chloroform concentrations should correlate, at least qualitatively, with higher total chloroorganics). In addition, chloroform can be rapidly and inexpensively analyzed to ppb levels using standard GC techniques, making it suitable for long term monitoring of the chloroorganic problem in a routine fashion.

The only known chloroform data for the St. Louis River prior to the start up of the WLSSD treatment plant is shown in Table 3. In general there is a paucity of data on chloroorganic levels in the estuary prior to the WLSSD start-up in 1979.

With the diversion of the Cloquet paper mill effluent to the WLSSD treatment plant in 1979, the chloroform levels in the St. Louis River,

TABLE 3

CHLOROFORM DATA ON THE ST. LOUIS RIVER PRIOR TO THE START-UP OF THE WLSSD TREATMENT FACILITY.[a]
SPRING 1978

Sample Location[b]	ug/l $CHCl_3$
Influent 1, Duluth Sewage Treatment Plant	4.7
Effluent 1, Duluth Sewage Treatment Plant	19.3
RR Bridge	1.9
Arrowhead Bridge	3.3
Oliver Bridge	5.1
Fond du Lac Bridge	7.5
Fond du Lac Dam	7.0
Forbay Lake (lower gate)	11.7
Forbay Lake (upper gate)	13.0
Highway 35 Bridge	17.3
Scanlon Dam	19.1
RR Bridge below Conwed	0
Highway 33 Bridge	0
Cloquet River	0
St. Louis River (Brookston)	0

[a]This is the only known background data on chloroform levels for this area. All of these samples were obtained on 5/3/78 with a water temperature of 12-14° C. Analysis done by Paula Johnson, UMD

[b]Check maps in Appendix A for sample sites.

between Cloquet and the mouth, have decreased markedly as illustrated in Table 4. It should be noted, however, that the results indicate that zero levels of chloroform in the upper St. Louis River have not yet been attained. This suggests that chloroform, or more likely chloroform precursors, that were absorbed into river bottom sediments during the time that the paper mill discharged its effluent into the river, are being released. Future studies should include analysis of these sediments.

Table 4 summarizes chloroform levels of sites sampled in three consecutive years, one prior and two subsequent to the start up of the WLSSD treatment plant. Table 5 shows chloroform data obtained in the summer of 1980.

At the same time, it should be noted that chloroform levels of the effluent being released into the harbor have increased dramatically (Table 4). Table 6 shows representative chloroform data for the WLSSD treatment plant and Table 7 shows chloroform concentrations at several locations within the plant itself. It is interesting to note that while the present chloroform levels of the Duluth city influent remain similar to the 1978 levels of the old treatment plant, the final effluent discharged into the harbor has increased by several orders of magnitude.

This increase is attributed to the diversion of the Cloquet pulp mill effluents to the WLSSD plant as illustrated by data in Table 8, which show the high levels of $CHCl_3$ originating from the Cloquet Pumping Station. These data imply that the point source of chloroform originally near Cloquet has not been eliminated but rather diverted, at least in part, to the Duluth-Superior harbor. These data certainly suggest that the WLSSD advanced secondary treatment facility is far from 100% efficient in removing chloroorganics.

Chloroorganics in Fish. By using electron capture detector (ECD) gas chromatography and GC/MS techniques several chlorinated pesticides and PCBs commonly found in Lake Superior fish were identified along with the other chlorinated compounds listed in Table 9.

Pentachlorophenol. From the ECD chromatograms, pentachlorophenol (PCP) was identified in the WLSSD fillet (Figure 6) but not in the Fond du Lac fillet (Figure 7). The identification of PCP was confirmed using GC/MS after further concentrating the fish extract. Pentachloroanisole was also identified in the WLSSD fish fillets. It remains to be determined, whether PCP or some other chloroorganic is the actual cause of tainting in the WLSSD fillet.

PCP and its sodium salt (Na-PCP) are among the most widely used biocides in the U.S. PCP is registered by the U.S. Environmental Protection Agency (EPA) for use as an insecticide (termicide), fungicide, herbicide, algicide, disinfectant, and as an ingredient in antifouling paint.

TABLE 4

CHLOROFORM DATA OF SELECTED SITES 1978-80[a]

Sample location[b]	Spring 78	Spring 79	Spring 80
Scanlon Dam	19.1	0.7	0.8
Fond du Lac	7.5	1.0	0.7
Oliver Bridge	5.1	1.0	0.4
Arrowhead Bridge	3.3	1.0	0.7
R.R. Bridge	1.9	---	3.7
Duluth Influent	4.3	---	5.6
Treatment Plant Effluent	18.9	---	138.8*

[a]example chloroform data, analysis by Paula Johnson and Bill Doucette.

[b]See maps in Appendix for samples sites.

*effluent from WLSSD treatment plant.

TABLE 5

SUMMARY OF CHLOROFORM DATA SPRING-SUMMER 1980[a]

Sample location[b]	$CHCl_3$ ppb(ug/l)
WLSSD	22.3
NSS	4.2
RR Bridge	3.7
Blatnik Bridge	2.9
Connors Pt.	2.9
Airport	1.8
Duluth Entry	0.6
Arrowhead Bridge	0.7
Oliver Bridge	0.4
Fond du Lac	0.7
Jay Cooke Park	0.7
Thompson Dam	0.7
Scanlon Dam	0.8

[a]average of 3 samples taken between 4-28-80 and 6-18-80, analysis done by Bill Doucette.

[b]See maps in Appendix A for sample sites.

TABLE 6

REPRESENTATIVE CHLOROFORM DATA FOR THE WLSSD TREATMENT[a] PLANT FOR THE MONTH OF JULY IN THE SUMMER OF 1979

Date	Flow in MLD (million liters/day)	Cl_2 Demand in mg/l	Plant Effluent in ppb $CHCl_3$
7/3	260	2.5	41.0
7/9	180	6.2	46.8
7/11	184	14.6	154
7/18	160	11.0	172
7/26	160	21.8	145

TABLE 7

AVERAGE CHLOROFORM CONCENTRATIONS AT SAMPLE POINTS[a] WITHIN THE WLSSD TREATMENT PLANT (11 sample dates 6-15-79 thru 1-11-80)

Sample point	$CHCl_3$ ppb
Duluth Influent*	5.6
Total Plant Influent	244.0
Secondary Effluent	169.0
Final Effluent Prior to Dechlorination	169.6
Final Effluent	138.8

[a]analysis by Paula Johnson, UMD.

*average of two samples.

TABLE 8

CHLOROFORM CONCENTRATIONS FOUND IN SAMPLES COLLECTED ON JANUARY 14,1980[a]

Sample	$CHCl_3$ ppb
Cloquet Pumping Station Effluent*	2990
Total Plant Influent	990
Duluth Influent	4.8
Final WLSSD Effluent	180
Breakwater site	108

*Combined effluents from the City of Cloquet, Potlatch paper mill, and Conwed wood products.

[a]analysis done by Martha Lewerenz, UMD.

TABLE 9

CHLORINATED ORGANIC COMPOUNDS IDENTIFIED IN FISH FILLETS BY GC/MS AND ECD GAS CHROMATOGRAPHY

WLSSD fillet

Compound	Identification[a]
Tetrachloroethylene	A
Chloro(chloromethyl)(1-methylethenyl)benzene	C
3,4-dichloro-2-propylphenol	C
Pentachlorophenol	B
Pentachloroanisole	C
Chlordane	A
Nonachlor	A
DDE	B
PCBs	B

Fond du Lac fillet

Compound	Identification[a]
Tetrachloroethylene	A
Chlordane	A
Nonachlor	A
DDE	B
PCBs	B

[a]A, identification based on MS; B, identification supported with retention time (EC/GC); C, fragmentation suggests a compound containing groups indicated.

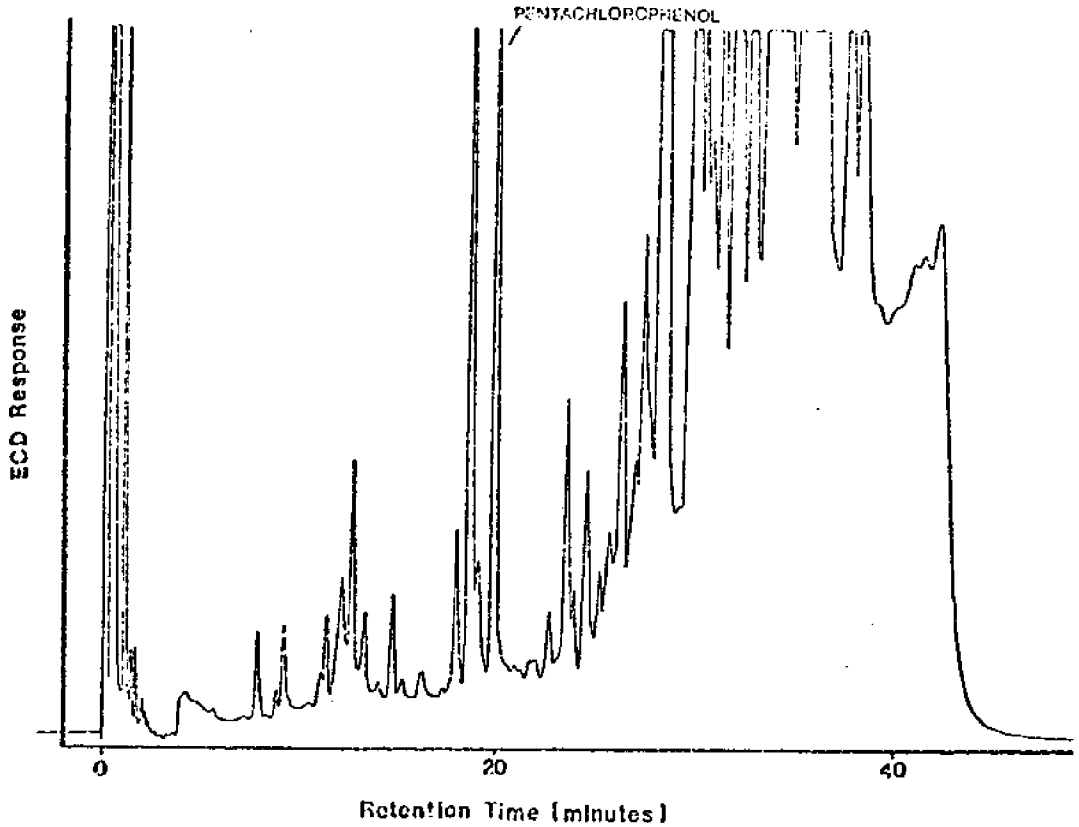


Figure 6 Electron Capture Detector chromatogram of WLSSD fish fillet extract.

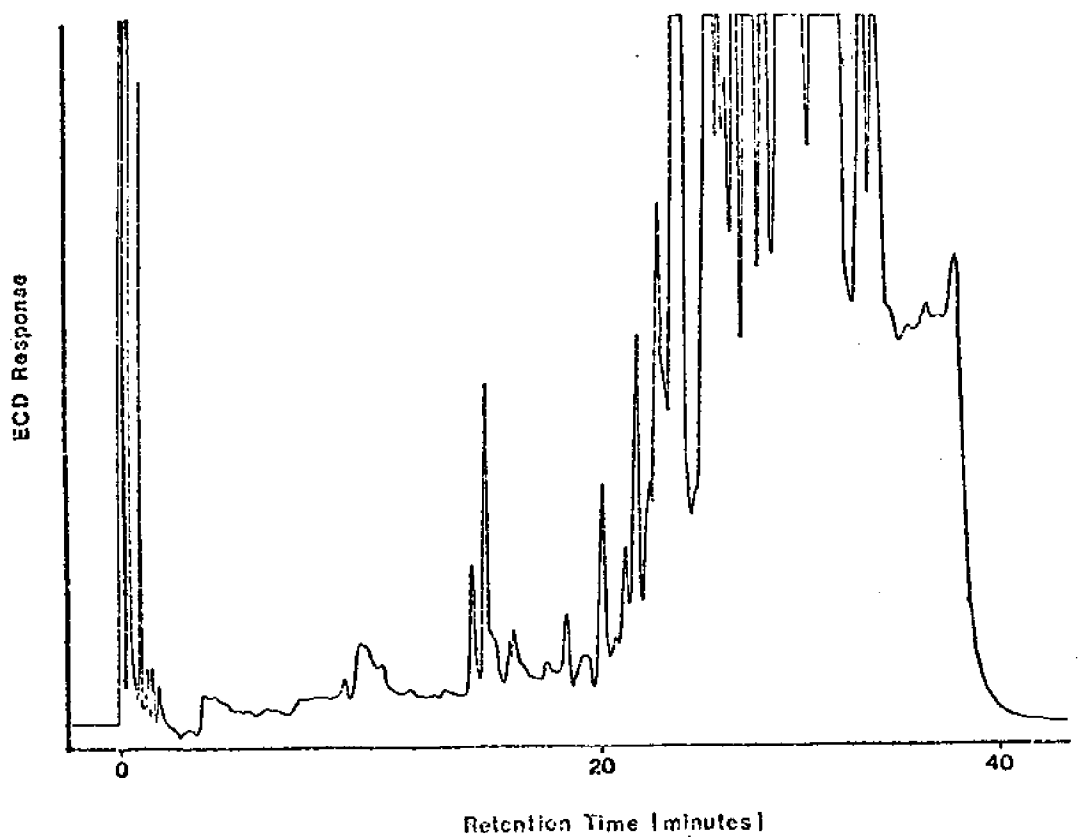


Figure 7 Electron Capture Detector chromatogram of Fond du Lac fish fillet extract.

Technical grade PCP also contains 4 to 12% tetrachlorophenols. Na-PCP is used as a slimicide and defoaming agent in the manufacture of paper and paperboard (Cirelli, 1978). Thus, contamination from many sources was possible. Both the WLSSD and Cloquet pumping station effluents, which contain effluents from Potlatch paper mill, Conwed wood products industry, and the City of Cloquet, Minnesota, were later analyzed in an attempt to identify any chlorophenolics originating there. The levels of chloroform near the WLSSD plant were much higher than the Fond du Lac or recovery site implying that there may be a correlation between $CHCl_3$ levels and the tainting of fish (Table 10).

Chloroorganics In Water

Palatability of fish in the St. Louis River has improved markedly since the diversion of the paper mill effluents to the WLSSD plant. Since previous studies have shown paper mill effluents to cause tainting of fish (Baldwin, 197 ; Gordon, 1980; Shumway, 1971) with low concentrations of chlorinated phenols implicated in the tainting (Burttschell, 1959), it was felt that the source of the tainting of the WLSSD fish may be the chloroorganics in the paper mill effluent not effectively removed or degraded by the treatment plant. Thus, the effluents from the WLSSD and Cloquet Pumping Station were examined for possible odor and taste causing chloroorganics.

Several compounds previously reported in pulp mill effluents were also identified in both the Cloquet Pumping Station and WLSSD effluent along with the WLSSD breakwater site. Among those identified were chlorinated phenols and the tri-and tetrachloroguaiacols as shown in Table 11. Pentachlorophenol was found in both effluents although it is not thought to be a product of the bleaching process.

In correspondence with Potlatch Corporation it was found that a slimicide containing 24.7% Na-PCP and 12% sodium salts of other chlorophenols, was used at an application rate of seven gallons per day. It was found through personal communication, that the two other major wood products industries whose effluents are treated by the WLSSD facility do not use PCP or NaPCP, although Superwood of Duluth had used PCP as a slimicide in the past.

It is interesting to note that no chloroorganics were identified in the Fond du Lac water sample which was analyzed by the same method as the effluent samples.

Table 12 shows the levels of $CHCl_3$ found at the four sample sites listed in Table 11. It appears that there may be some type of qualitative correlation between chloroform levels and the number of chloroorganics identified in the water.

Since the chloroorganics were identified in both the Cloquet Pumping

TABLE 10

FISH STUDY CHLOROFORM DATA[a]

	$CHCl_3$ ppb					
Sample location[b]	4-28-80	4-29-80	5-2-80*	5-4-80	5-6-80	average
WLSSD	10.2	12.6	45.7	---	---	22.3
NSS	2.7	3.6	6.2	---	---	4.2
Connors Point	1.7	2.7	4.2	---	---	2.9
Airport	1.1	---	2.6	---	---	1.9
Arrowhead	0.8	---	0.7	0.8	0.8	0.8
Fond du Lac	0.8	---	0.8	---	---	0.8

[a]analysis done by Bill Doucette and Paula Johnson.

*high flow received by WLSSD from Potlatch.

[b]See map in Appendix A for locations.

TABLE 11

CHLORINATED ORGANIC COMPOUNDS IDENTIFIED IN WLSSD AND CLOQUET PUMPING STATION EFFLUENTS BY GC/MS AND ECD GAS CHROMATOGRAPHY*

Compound	Identification[a]
Dichlorophenol	A
Trichlorophenol	B
Tetrachlorophenol	B
Pentachlorophenol	B
Trichloroguaiacol	A
Tetrachloroguaiacol	A

*The same compounds were identified in the WLSSD breakwater site. No chlorinated compounds were identified in the Fond du Lac water sample.

[a]A, identification based on MS; B, identification supported with retention time (EC/GC).

TABLE 12

CHLOROFORM CONCENTRATIONS AT SITES LISTED IN TABLE 11

Sample site	ave. $CHCl_3$ ppb[a]
WLSSD Effluent	180
Cloquet Pumping Station Effluent	3000
WLSSD Breakwater site	108
Fond du Lac	0.8

[a]Average of two samples.

Station and WLSSD effluents it is likely that their origin is the Potlatch paper mill in Cloquet. It is also thought that while chloroform and other chloroorganics are produced during the chlorination at the WLSSD water treatment facility, the main source of chloroorganics in the WLSSD effluent is the Potlatch paper mill. Thus, the WLSSD treatment plant is not completely effective in eliminating the chloroorganic compounds from the Cloquet effluent.

From the results, it is felt that chloroform may be a possible indicator of the chloroorganic problem associated with the paper mill effluent. However, chloroform may not be a good indicator of a pentachlorophenol (PCP) problem since PCP is not thought to be formed during the bleaching process. Also since paper mill effluents have been shown to be responsible for tainting of fish and since the chlorinated phenols in these effluents are thought to be the cause, it is likely that $CHCl_3$ monitoring can be used to predict possible taste and odor problems occurring in fish living in the St. Louis River.

Analysis of Sediment Sample

A harbor sediment sample taken near the WLSSD treatment plant showed the presence of several chlorophenols. The sediment was analyzed using steam distillation, CsOH/Silica gel chromatography and GC/MS techniques with the compounds identified listed in Table 13. Further sampling and analysis of St. Louis River sediments needs to be done to determine the extent of chloroorganic contamination and its possible long range effects on the river ecosystem.

TABLE 13

CHLORINATED ORGANIC COMPOUNDS IDENTIFIED
IN WLSSD SEDIMENT SAMPLE*

Compound	Identification[a]
pentachlorophenol	B
trichlorophenol	C

[a]A, identification based on MS; B, identification supported with retention time; C, fragmentation suggests a compound containing groups indicated.

*See map in Appendix A for sample location.

Fish Tainting Study

In an attempt to evaluate the impact of diverting the Potlatch paper mill effluent to the WLSSD treatment facility, a preliminary study was done at the end of April, 1980 to determine the possible effect, if any, that the WLSSD effluent would have on fish residing in that area. The sites were chosen (Fond du Lac, the Arrowhead Bridge, and the area directly in front of the WLSSD outfall) on the basis of anticipated differences in water quality. The redirecting of the paper mill wastes from the upper portion of the estuary to the WLSSD located in the lower portion of the harbor should lead to a dramatic improvement in the consumer acceptability of those fish that reside away from the WLSSD discharge.

Thirty five walleye were obtained from the Minnesota and Wisconsin Department of Natural Resources (DNR) on April 28, 1980 during DNR's annual walleye electro-fishing and tagging procedure in the St. Louis River near Fond du Lac, Minnesota. The plan was to take the walleye from the upstream area (Fond du Lac) and transfer them to live nets held proximate to the WLSSD for 5 days and then take a portion of these fish to a recovery area (Arrowhead Bridge) for an additional 5 days. The fillets from fish taken from the three sites would then be compared in a standard triangular testing regime (ie. 1 piece of one-type; 2 pieces of another; then choose the one of the three that is different, not based on demonstrated ability to discern differences in taste and odor). The tasting panel of eight members included students and staff from UMD Chemistry Department and a staff member of the Duluth Herald and News Tribune.

The fish were baked with no application of salt, spices, or oil and were served in paper "butter cups" on eight inch paper plates. The samples were identified with randomly chosen three digit numbers. The participants were asked first to smell, and then to taste the samples. They were also requested not to eat the samples and to rinse their mouths with water between tests. The testing was conducted in the UMD Home Economics Department in a room removed from the area where the cooked fish were prepared.

The "Summary of Taste Panel Results" (attached) indicates a clear preference for Fond du Lac Fish over either the WLSSD or the "recovered" samples. It also appears that there is no clear differentiation between WLSSD and "recovered" samples. The results thus demonstrate the improvement in fish taste in the upstream areas of the St. Louis River because of the initiation of wastewater treatment by the WLSSD. The results may also suggest that if the fish for some reason, become tainted from the WLSSD discharge that the "recovery" period might be prolonged.

Spring 1980
St. Louis River Study
Summary of Taste Panel Results (2 Sets of 3 Samples)

Comparisons:	"Which Sample Do You Prefer?" Odor	Taste
I. WLSSD vs.	--	--
Fond du Lac	XXX	XXX
"No Difference"	XXX	XXX
II. WLSSD vs.	X	X
"Recovered" Fish	--	--
"No Difference"	XXXX	XXX
III. Fond du Lac vs.	XX	XXX
"Recovered" Fish	---	---
"No Difference"	XXX	XX

Taste Panel Members

1. Mr. David Peake
2. Mr. David Hewetson
3. Dr. Ronald Caple
4. Mr. Mark Deeg
5. Mr. Todd Swanson
6. Mr. Greg Mix
7. Dr. Larry Thompson
8. Mr. Doug Smith

Furthermore a qualitative fish flavor survey was made of recreational fishermen in the St. Louis River in the Spring of 1980. Approximately fifty self-addressed and stamped questionnaires (See Figure 8) were handed out to persons fishing in the St. Louis River and harbor. Twenty completed forms were returned and the results are summarized below:

Years fished in St. Louis: 45% of those responding had fished 6 or more years while 80% had fished at least 3 years.
Kinds of fish caught: northern pike, yellow perch, walleye
Where caught? Various locations in the St. Louis River with Arrowhead Bridge and Pokegamma Bay mentioned most frequently.

Flavor problems:	Yes	No	No opinion
At present	26%	74%	
Any time	75%	15%	10%

Of the people responding yes to the flavor problem question, 86% said that the problem was prior to 1979 while 14% said that there has always been a flavor problem.

Although hampered by a limited number of responses, the survey does seem to correlate the improvement of the flavor quality with the diversion of the Cloquet pulp mill effluent to the WLSSD plant and also with the decreased $CHCl_3$ levels in that section of the river.

With the diversion of the effluent to the WLSSD plant and increasing levels of chloroform found in the WLSSD effluent and in various harbor sites, it is thought that while the flavor impairment may be reduced inward of the harbor, a potential problem remains because the source of the problem, at least in part, has only been relocated downstream in the harbor area.

NEW PHENOL ANALYSIS USING 2-FLUORENYL SULFONATES

A phenolic compound may be defined as any aromatic molecule possessing one or more hydroxyl substituents and it may be derived from synthetic or natural sources. The bulk of the synthetic phenols are produced from such sources as gas works and oil refineries, the coking of coal, chemical plants, wood preserving operations, and the manufacture of dyes.

Other phenolics are the result of chemical and biological oxidation of aromatic compounds present in the environment, and are primarily generated from the decomposition of aquatic and terrestrial vegetation (lignin sources). Moreover, chemical processes such as water treatment with chlorine, may result in the chlorination of phenolic materials already present in the waters. An indication of their widespread industrial use is given in Table 14.

Phenols in industrial effluents are said to be "among the most injurious and far-reaching of toxic agents entering the aquatic environment"

SPRING 1980

FISH FLAVOR SURVEY

This survey is designed to determine the general satisfaction with the flavor of the fish in the Duluth-Superior Harbor and the St. Louis River. Your help would be appreciated.

Please circle or fill in the appropriate answer.

1) What kind of fish did you catch (eat)?

northern pike yellow perch walleye other _____

2) At what location was this (these) fish caught?

3) Did you notice any fish with what you considered poor flavor?

Yes No

4) If you answered yes to question 3, how many fish did you catch? ____

How many of the fish had a bad flavor? ____

5) How many years have you fished in the Duluth-Superior Harbor or the St. Louis River?

1 2 3 4 5 6 or more

6) For how many years have you eaten fish from these areas?

1 2 3 4 5 6 or more

7) If you fished the harbor or river in past years, have you ever noticed any flavor problems? When?

Yes No

8) Have you noticed any improvements in flavor in the past year?

Yes No

9) How do you feel that the flavor of the fish in the harbor or river compares to the flavor of the same species caught in other areas?

Better Worse About the same

Comments (Please make any specific comments which you feel may be helpful)

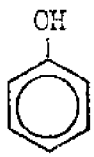
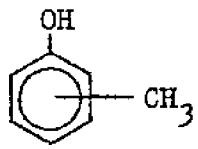
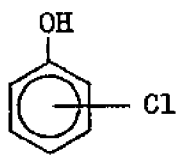
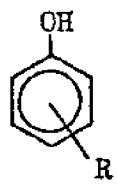
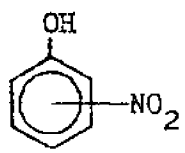
Please return as soon as possible to:

Bill Doucette
Chemistry Department
UMD
Duluth, MN 55812

Figure 8 Fish flavor survey distributed in Spring 1980.

Table 14

Various Phenolic Compounds and Their Uses (Buikema et al., 1979)

Class of Compound	Structure	Source/ Synthesis	Uses
Phenol		Cumene Benzene	53% phenolic resins 8% bisphenol A 7% alkylphenols 7% caprolactam 25% other
Cresols		Petroleum or coal tar	28% phenolic resins 25% tricresylphosphate 10.7% disinfectants 8.9% antioxidants 8.4% engine and metal cleaners 7.1% ore-flotation 6.2% wire-enamel solvent 4% miscellaneous
Chlorophenols		Phenol Chlorobenzene Nitrobenzene	Biocides and intermediates for biocides; Wood preservation
Alkylphenols		Phenol	Antioxidants (BHT) Gasoline, oil Greases Plastics
Nitrophenols		Phenol Nitrochloro-benzene Benzene	Dyestuff Explosives Intermediates

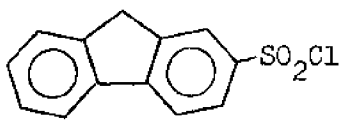
(Stolbunov et al., 1976). While human illness attributable to these materials is not common, episodes of phenol poisoning have occurred, usually following industrial exposure. Symptoms include diarrhea, dark urine, mouth sores, and skin rash. These symptoms occurred in persons exposed to carbolic acid (i.e. phenol) in their water supply at concentrations >0.1 mg/liter. In terms of their toxicity, phenols are "structurally nonspecific". That is, they produce a common biological effect that is not dependent on the exact molecular structure. Rather, their accumulation in some vital loci within cells, causes interference in fundamental metabolic processes (Blackman et al., 1955). The U.S. Environmental Protection Agency (EPA) has set a maximum limit for phenol in drinking water as 0.001 mg/liter (Kuehl et al., in press).

The present report discusses a sensitive new technique that has been developed (MS Thesis, Todd Swanson, UMD, 1980) for the analysis of phenolic compounds in natural waters. The analytical procedure utilizes 2-fluorenyl sulfonyl chloride as a fluorescent "label." The phenols are allowed to react with the sulfonyl chloride, and the labeled compounds are subsequently analyzed by high-performance liquid chromatography (HPLC) employing fluorescence detection. Use of the fluorenyl derivatives and fluorescence detection have lowered the detection limits about fifty times over conventional UV detection systems (a few micrograms per liter, without sample cleanup or pre-concentration). A typical phenol determination requires about two hours from start to finish.

In recent years, High-Performance Liquid Chromatography (HPLC) has become an extremely important separation technique for the analysis of complex organic mixtures. Since the analytical problem is often one involving trace quantities of materials in complex matrices, the need has arisen for detector systems that are more suitable than the commonly used refractive index or UV detectors. For this reason fluorescence detection, which is both sensitive and selective, is being used increasingly for the analysis of complex biological and environmental samples.

To detect non-fluorescent molecules, or those which only weakly fluoresce, techniques have been developed which enable the introduction of a fluorescent label. The best of these labels are those which are stable,are specific for the compound type in question, and have high quantum yields and extinction coefficients. For derivatization and the subsequent isolation and for "reverse-phase" HPLC analysis, it is also desirable to have moderate solubility in the derivatizing agent and only slight water solubility in the product. The goal in the present investigation is to encompass all of these desirable characteristics in a new fluorescent label for the analysis of trace levels of environmentally important phenols. The successful candidate that passed all

our stringent criteria was 2-fluorenyl sulfonyl chloride.



2-Fluorenyl Sulfonyl Chloride

EXPERIMENTAL

Materials. Fluorene, phosphoryl chloride, chlorosulfonic acid, and p-cresol were obtained from Eastman Organic Chemicals. Phosphorous pentachloride was obtained from the Baker Chemical Company. Triethylamine, o-chlorophenol, quaiacol, p-chlorophenol, 2,6-dichlorophenol, 2,4-dichlorophenol, and pentachlorophenol were purchased from the Aldrich Chemical Company. Sulfuric acid was obtained from Hi-Pure Chemicals. Potassium and sodium hydroxides were obtained from Matheson Coleman/Bell. Sodium carbonate, magnesium sulfate, phenol and chloroform (analytical reagent) were purchased from Mallinckrodt Chemical Company. m-Cresol, hexane (analytic reagent) and acetonitrile (HPLC grade) were obtained from Fisher Scientific Company. Methylene chloride (technical grade) was obtained through the University of Minnesota chemical storehouse.

The distilled water used in the HPLC analysis was passed through a Filterite model #LNO 10B-3/8 canister, a Continental deionizer, and two 7x600 mm Bondapak C-18® Porasil B reverse-phase HPLC columns (Waters Associates).

Apparatus. The following HPLC apparatus was manufactured by Waters Associates: two M-6000 pumps, a model 660 solvent programmer, a model U6K injector and a model 440 dual channel UV detector equipped to monitor 254, and 280 nm. The analytical column material was 10µm HC-ODS® (0.26x25cm). Chromatograms were recorded on a dual pen recorder (Texas instruments) or a Hewlett Packard 3380S recorder/integrator.

The fluorescence spectra were obtained using a 650-10S Perkin Elmer spectrophotometer equipped with excitation and emission monochrometers and standard 1 cm quartz cells. This same instrument, equipped with a 28µl flow cell, was used as the fluorescence detector in the HPLC work.

The following instruments were used for obtaining other spectral data: an EM360 NMR Spectrometer, a Beckman DK-2A UV-Visible spectrophotometer and a Beckman IR33 for obtaining infra-red spectra.

Environmental water samples were stored in 100 ml glass bottles with teflon septa. The samples were filtered through GF/F Microfibre® filters (0.7µ) prior to analysis.

Procedure for Preparation of the Derivatizing Agent (Scheme 1) 33.3 g (0.20 moles) fluorene is dissolved in 400 ml of methylene chloride contained in a 1 ℓ round bottom flask. A 100 ml solution of 13.2 ml (0.20 moles) chlorosulfonic acid ($ClSO_3H$) in methylene chloride is added slowly, with stirring, over a 2 hour period. The reaction vessel is kept below 5° C with an ice bath. The product is filtered off and washed with hexane to give crude fluorene-2-sulfonic acid in 74% yield.

The fluorene-2-sulfonic acid is dissolved in water and converted to the potassium salt by the addition of 1 M KOH until basic. The product precipitates as a white solid and the slurry is stirred for 1 hr. The potassium fluorene-2-sulfonate is filtered, washed with ether and dried over P_2O_5 in a vacuum dessicator. (79% yield).

23.2 g (0.083 moles) potassium fluorene-2-sulfonate and 23.8 g (0.11 moles) PCl_5 are placed in a 1 ℓ round bottom flask. A 100 ml solution of $POCl_3$ (12 ml, 0.13 moles) in chloroform is added over 10 min., with stirring. 300-400 ml chloroform is added to the reaction vessel and the contents refluxed for 3 hours. The mixture is filtered to remove salts and the filtrate washed with 3-80 ml portions of water and dried over Na_2SO_4. The solvent is evaporated in vacuo to give 80-90% crude fluorene-2-sulfonyl chloride. The product is recrystallized from chloroform. (mp. 162-164°)(Janczewski et al., 1964; Chrzaszczewska et al.,1967).

Procedure for Preparation of Phenol Derivatives.(Scheme 2 + Table 15) Two eq. of the phenol are mixed with 1 eq. of fluorene-2-sulfonyl chloride with enough methylene chloride added to produce a slurry. Reaction occurs upon addition of 4 eq. of triethylamine to the slurry.

The reaction mixture is diluted with methylene chloride, and washed successively with 10 ml portions of water, 2 x 1M NaOH, 2 x 1M H_2SO_4, brine, and water until reaching a neutral pH. The organic layer is dried with anhydrous $MgSO_4$ and the solvent evaporated in vacuo to give the derivatized phenol in 85-95% yield. Recrystallization is performed from methylene chloride/hexane.

Preparation of a Standard Curve. Mixed standard solutions of the eight previously prepared phenol derivatives were made at four different concentrations. Solutions of concentrations 20, 60, 100, and 200 ng/ml in each derivative were prepared in acetonitrile. 30 µl injections of each solution were made in triplicate, with peak areas determined using a Hewlett Packard 3380S recorder/integrator.

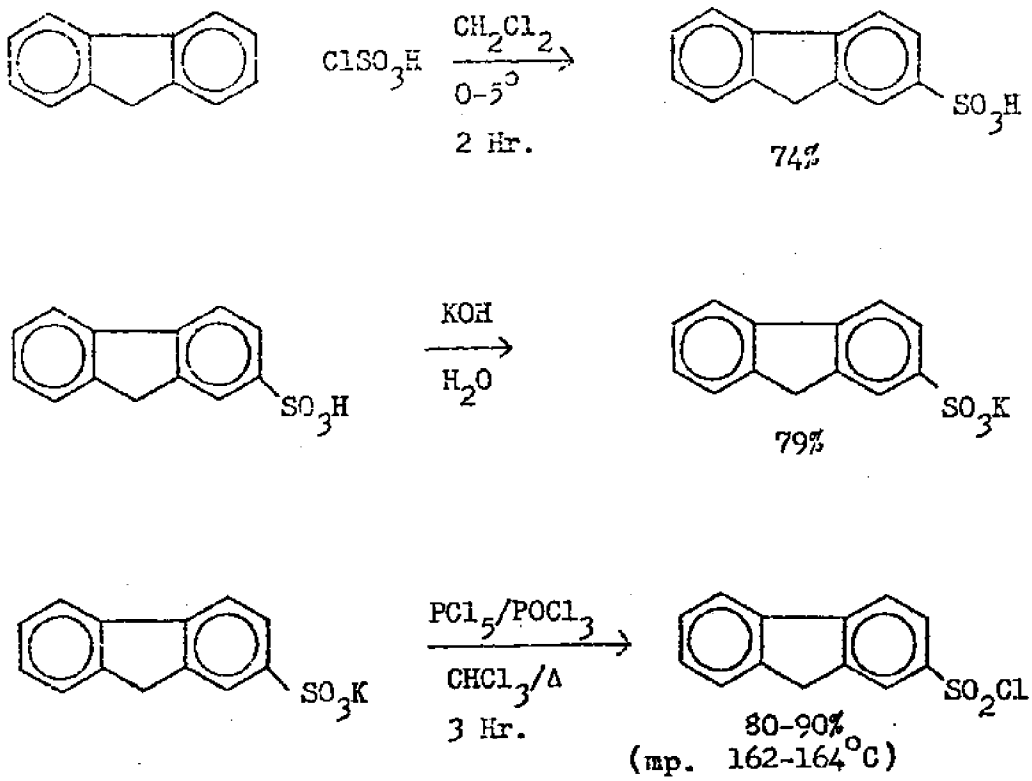
Procedure for Phenol Derivatization in Aqueous Media. The aqueous sample is filtered through GF/F (0.7 µm) Microfibre^R filters. 9.0 ml of the aqueous sample is transferred to a 25 ml round bottom flask. The pH is adjusted to 8.5 with Na_2CO_3. To this solution are added 1 ml acetonitrile and 2 ml of the reagent solution (100 mg fluorene-2-sulfonyl chloride in 100 ml acetonitrile). The flask is fitted with a

condenser and the contents stirred at 55° for 1 hour. Hydrolysis of excess reagent is then accomplished by adjusting the pH to 9.5-10 with additional Na_2CO_3 and continued heating for 1/2 hour. The reaction mixture, when cooled and made slightly acidic (pH-6) with 3.6 M H_2SO_4, is ready for HPLC analysis.

Scheme 1

Preparation of the Fluorescent "Label":

2-Fluorene sulfonyl chloride (Janczewski et al.,1964; Chrzaszczewska et al., 1967)



Scheme 2

Preparation of "Standard Derivatives"

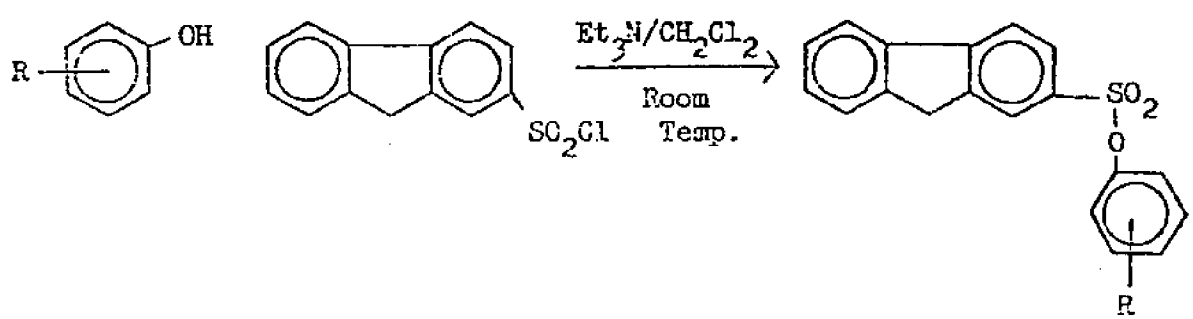
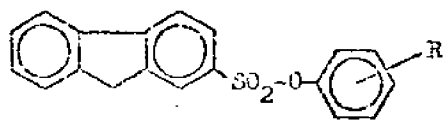


Table 15

"Standard" Derivatives



Phenol	Derivative formula	% yield	mp.	observed elemental analy. % C	%H	U.V. max	fluorescence ex.	em.
m-Cresol (R=m-methyl)	$C_{20}H_{16}O_3S$	89.0	95-96	71.32	4.82	282 293 304	280	325
p-Chlorophenol (R=p-chloro)	$C_{19}H_{13}O_3SCl$	88.9	116-116.5	64.02	3.70	282 293 304	280	325
2,4-Dichlorophenol (R=o,p-dichloro)	$C_{19}H_{12}O_3SCl_2$	94.4	151-151.5	58.40	2.92	282 293 304	280	325
p-Cresol (R=p-methyl)	$C_{20}H_{16}O_3S$	92.2	99.5-100	71.38	4.78	282 293 304	280	325
2,6-Dichlorophenol (R=o,o'-dichloro)	$C_{19}H_{12}O_3SCl_2$	81.0	171-172.5	57.25	3.07	283 294 305	280	325
Pentachlorophenol (R=pentachloro)	$C_{19}H_9O_3SCl_5$	90.8	200-201	45.42	1.87	284 294 306	280	325
o-Chlorophenol (R = o-chloro)	$C_{19}H_{13}O_3SCl$	90.1	110-111	63.94	3.72	282 293 304	280	325
Guaiacol R=(o-methoxy)	$C_{20}H_{16}O_4S$	97.6	127-128	68.26	4.72	281 293 304	280	325

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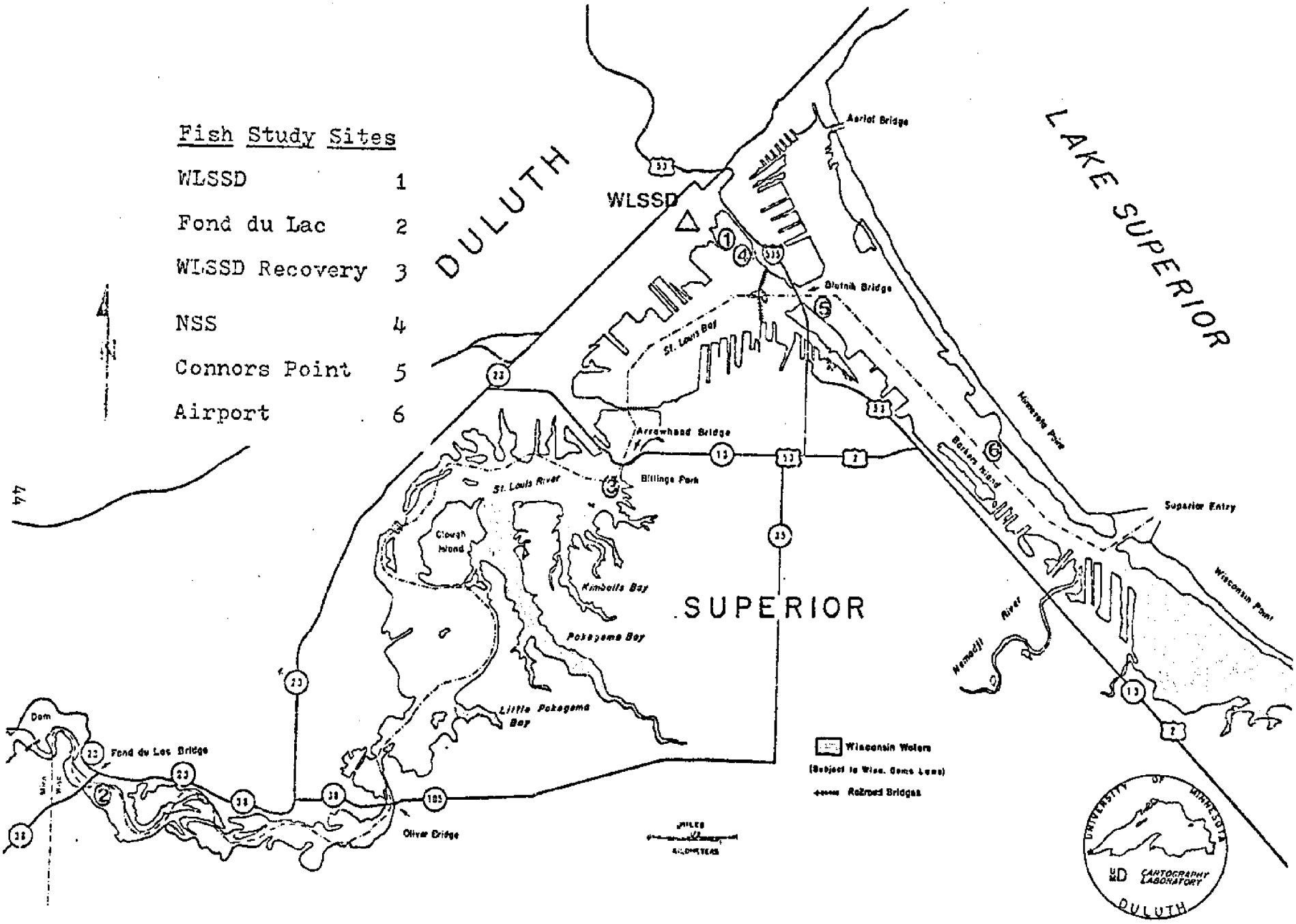
APPENDIX A

MAPS OF THE ST. LOUIS RIVER AND DULUTH-SUPERIOR HARBOR


Fish Study Sites
WLSSD 1
Fond du Lac 2
WLSSD Recovery 3
NSS 4
Connors Point 5
Airport 6
DULUTH
WLSSD
LAKE SUPERIOR
SUPERIOR
Aerial Bridge
Blatnik Bridge
St. Louis Bay
Arrowhead Bridge
Billings Park
St. Louis River
Clough Island
Kimballs Bay
Pokegema Bay
Little Pokegema Bay
Barkers Island
Superior Entry
Wisconsin Point
Nemadji River
Fond du Lac Bridge
Oliver Bridge
Dam
Wisconsin Waters
(Subject to Wisc. Game Laws)
Railroad Bridges
MILES
KILOMETERS
UNIVERSITY OF MINNESOTA
CARTOGRAPHY LABORATORY
DULUTH

Sample Sites-Table 3

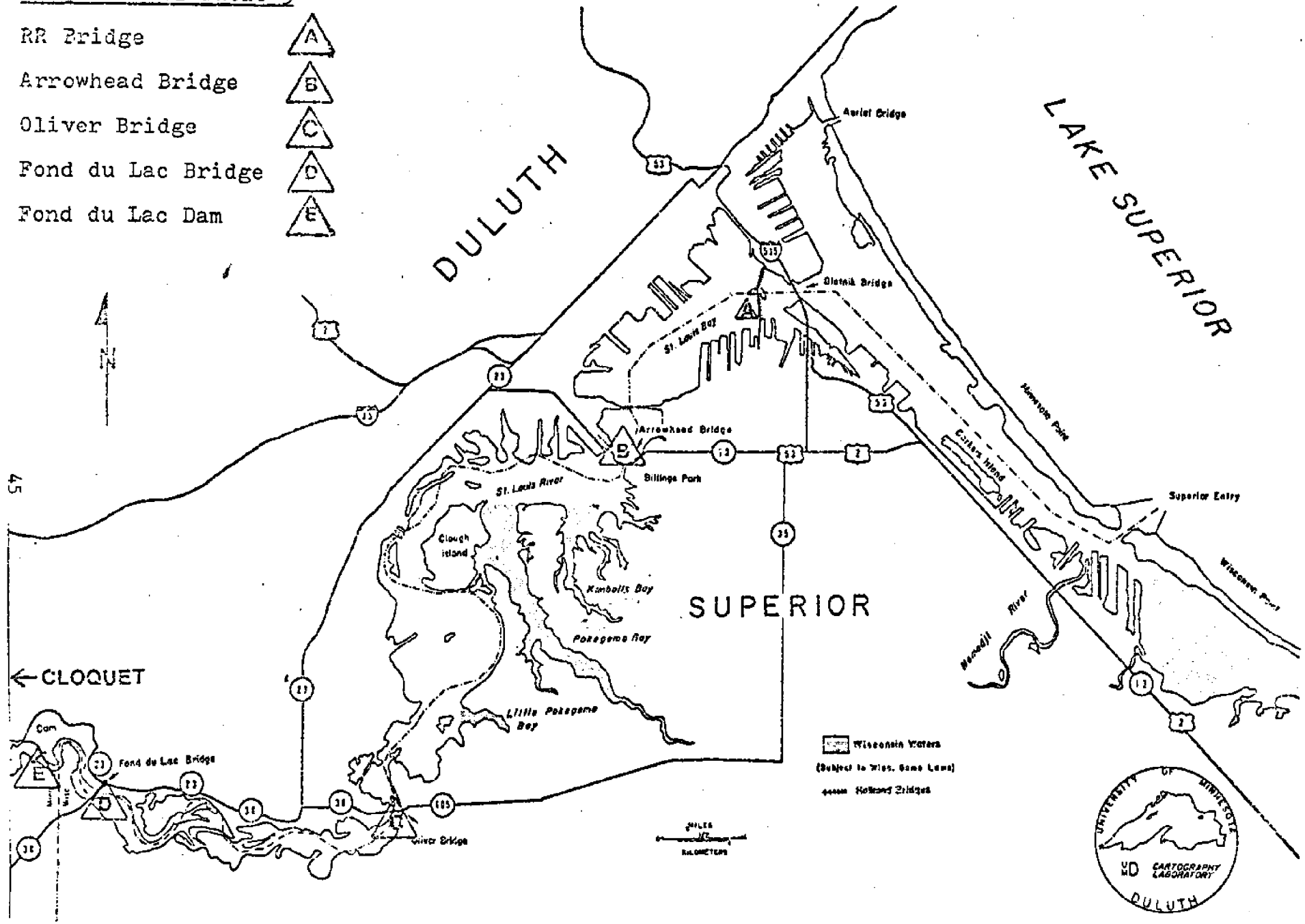
RR Bridge A

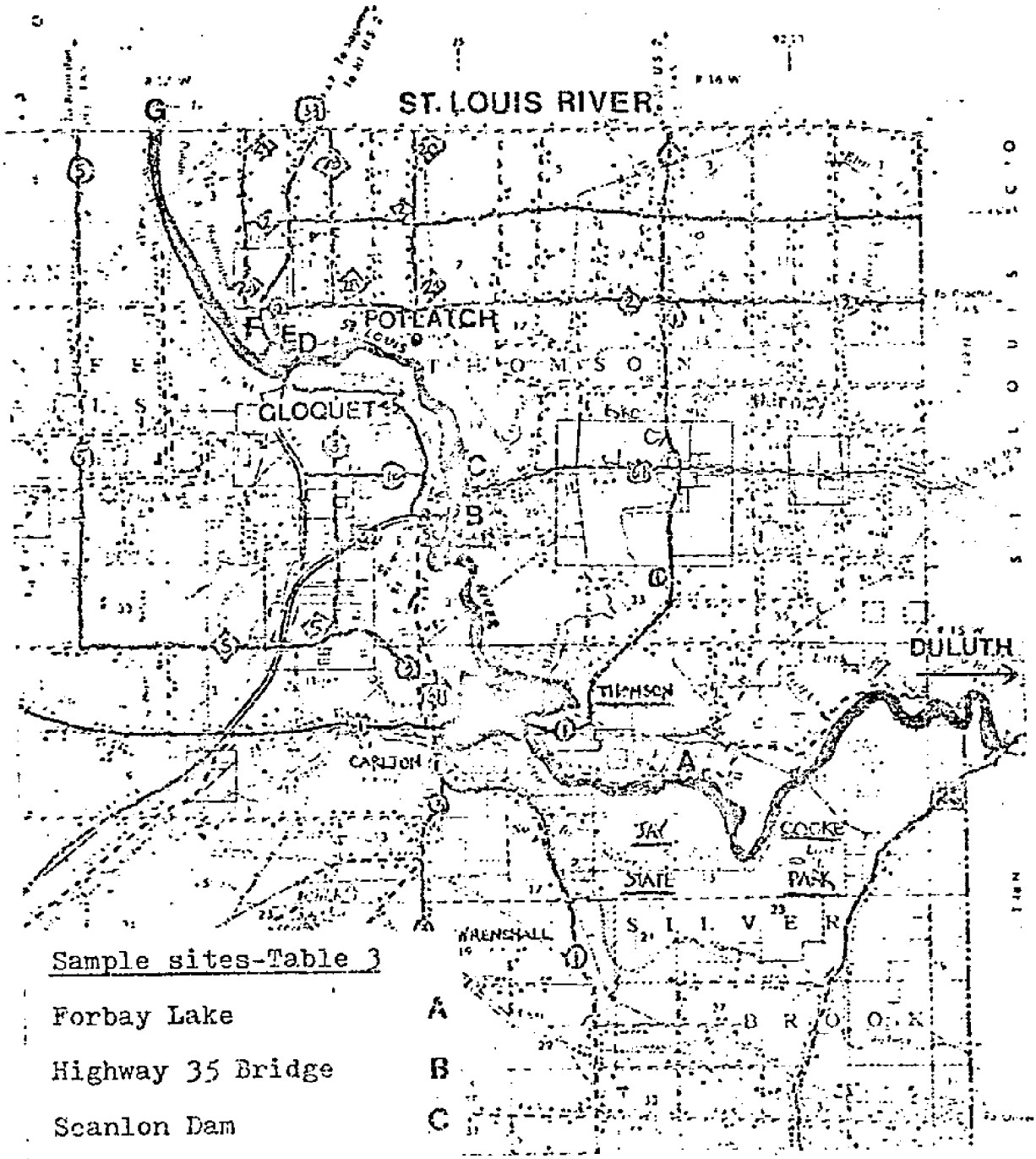
Arrowhead Bridge B

Oliver Bridge C

Fond du Lac Bridge D

Fond du Lac Dam E





Sample sites-Table 3

Forbay Lake	A
Highway 35 Bridge	B
Scanlon Dam	C
RR Bridge Below Conwed	D
Highway 33 Bridge	E
Cloquet River	F
Brookston	G

Sample Sites-Table 5

WLSSD	A
NSS	B
RR Bridge	C
Blatnik Bridge	D
Connors Point	E
Airport	F
Duluth Entry	G
Arrowhead Bridge	H
Oliver Bridge	I
Fond du Lac	J
Jay Cooke Park	K
Thompson Dam	L